Aus der Abteilung für klinische Immunologie des Universitätskrankenhauses
Erlangen-Nürnberg
(Vorstand: Professor Dr. med. F. Scheiffarth)

Die Unterdrückung der 19 S- und 7 S- Immunglobulinproduktion durch heterologe und isologe Immunseren

INAUGURAL-DISSERTATION

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Erlangung der Doktorwürde

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Suppression of 19S- and 7S-Antibody-Formation with Homologous and Heterologous Immunsera

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With 2 Figures

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Abstract

This work was designed to show whether heterologous and homologous antisera against SRBC are as efficient in suppression of 19S- and 7S-antibody-formation as isologous antisera. Immunsera were induced in rabbits, guinea-pigs, mice of the inbred strains C57Bl and AKR. AKR-mice were sensitized with SRBC. The immunsera were injected 1 day before and 1 day after sensitization. 10 days thereafter spleen cells producing LHG were counted. In the animals treated with immunsera an extreme depression of 19S- and 7S-antibody-formation was found. This effect was demonstrated with all immunsera of the different species. Controls injected with sera of unsensitized rabbits, guinea-pigs etc. showed only a slight inhibition of cellular antibody-formation. No influence was observed on the background after application of immunsera. The results show, that the specificity of immunsera is more important than their origin. This is the effect of the binding of the antigenic sites by the Fab-fragments.

The possibility to suppress the immune response by passive transfer of specific antibody revealed a feedback system (9). For a long time it also has been known that mixing of antigen with the antibody can prevent the primary response (7). The mode of action is thought to be a removal of antigenic determinants by the antibody. Thus the combination between antigens and receptors of the immunocompetent cells situated on the surface will be blocked. It is presumed that blocking of the antigenic sites, which is called the peripheric level, alone will not be sufficient for interpretation the possibility of inhibition of antibody-formation even if administered after induction of the antibody-synthesis (5). Moreover, some authors have suggested a direct action of the antibody at the cellular or central level (6). The specific action of the administered antibodies shows that the inhibitory capacity resides on the Fab-fragments. Therefore antibodies with high affinity will be more efficient inhibitors (8). It is not surprising that late 7S-antibodies are more inhibitory than early 7S-antibodies (2). The principle of the feedback-system implies that the specifity of the

antibodies is more important than their origin. This was to demonstrate using heterologous and homologous immunsera for specific inhibition of the cellular antibody-formation.

Material and Methods

To demonstrate the cellular antibody-formation inbred female AKR-mice were used. Each animal was at least 4 months old. The average bodyweight was 30 g.

Production of antisera against sheep-red-blood-cells (SRBC)

Rabbits, Purbright guinea-pigs, mice of the inbred-strains C 57 Bl and AKR were sensitized by i.p., i.m. or i.v. injections of SRBC. The antibody-activity of their sera was determined at regular intervals. At the peak of the titer the animals were killed and bled (mice were bled 8 days after i.p. injection of SRBC). The individual sera were pooled and stored at —20° C.

Influence of immunsera on the cellular antibody-formation

1 ml of 1 immunserum was injected i.p. into the AKR-mice twice with an interval of 1 day. During the day between the injections the AKR-mice were sensitized with 1 dose of 4×10^8 SRBC i.p. After 10 days the mice were sacrificed and the spleen removed. The cellular antibody-formation was determined by counting the plaque-forming cells (PFC) detected by the local hemolysis in gel (LHG) performed in the usual way (1, 3, 4). Each series receiving either serum of rabbits or guinea-pigs etc. consisted of 6 animals. The spleen of each animal was examined separately. The mean value of several tests was determined.

Control 1: Serum from unsensitized rabbits, guinea-pigs etc. was injected under the same conditions into AKR-mice sensitized with 4×10^8 SRBC i.p. These sera had no antibody-activity against SRBC.

Control 2: AKR-mice were sensitized with 4×10^8 SRBC i.p. but received no serum at all.

Control 3: Immunserum of rabbits, guinea-pigs etc. was i.p. injected to AKR-mice, which were not sensitized.

Each control series consisted of 6 animals too. The spleen of those animals was also examined separately. It must be stated, that in the 6 experiments performed each control-series was sensitized with the same suspension of SRBC as the animals of the main experiment.

Results

Cellular antibody-formation in sensitized animals without injection of serum

The AKR-mice showed a good reaction with more than 100 PFC per million spleen-cells. The counts of IPFC for each experiment exceeded the counts of DPFC as usually found 10 days after sensitization with SRBC in mice.

Cellular antibody-formation in sensitized animals after application of immunsera

Application of immunsera according to the above mentioned pattern considerably reduced the counts of PFC. Combining the results of the 6 experiments the number of 19S-immunglobulin-producing DPFC in treated animals varied only from 0.9 to 6.4% of those in only sensitized animals. Similar results were found in respect to the 7S-immunglobulin-producing IPFC in treated mice. No correlation between the antibody-activity of the immunsera and the inhibitory efficiency was observed. Also the genetic difference between the species that produced the immunserum and the AKR-mice was of no importance.

Cellular antibody-formation in sensitized animals after application of normal serum

Sensitized AKR-mice had, after application of serum without antibodies against SRBC, a diminished content of PFC. However, the inhibition was less than 50%. Contrary to this, animals treated with normal serum of C57Bl-mice showed an increase of DPFC as well as of IPFC. The increase was 11 resp. 13%.

Unsensitized animals after application of antiserum

There was no demonstrable effect on the "background". In single animals the counts of PFC were slightly increased.

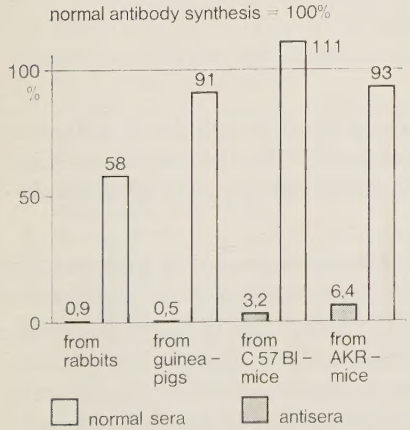

Fig. 1. Influence of antisera and normal sera of different species on the 19S-antibody-synthesis.

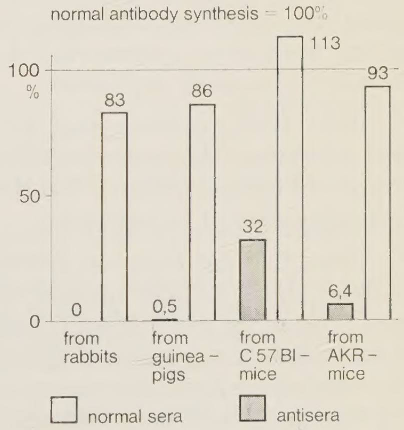

Fig. 2. Influence of antisera and normal sera of different species on the 7S-antibody-synthesis.

Figure 1 shows the degree of suppression of the cellular 19S-antibody-formation after application of immunserum of different species in comparison to the effect of normal serum.

Figure 2 shows the behaviour of 7S-immunglobulin-producing cells under the same conditions.

Discussion

The suppression of the cellular antibody-formation by application of heterologous and homologous immunsera has been demonstrated with certainty. In relation to the content of PFC in sensitized untreated animals the content was reduced markedly when antisera against SRBC had been applied. The objection that the results show only a nonspecific effect depending on the application of serum i.p. is not valid, because this effect is much stronger than the suppression by sera of unsensitized animals. The specific inhibition can be demonstrated with the immunserum of all species. Thus a general principle can be deduced. Heterologous antisera seemed to be more efficient than homologous and isologous antisera. The difference can be explained by the higher affinity of the "late" heterologous antibodies in comparison to the "early" homologous and isologous antibodies.

The results can be integrated completely into the conception of the regulation of the immune-response. The simplest interpretation is one of binding of antigenic sites by antibodies. They are hindered from being processed by immunocompetent cells. Thus the antibody-formation will be initiated only to a small extent. The quantity of antigen-binding antibodies was sufficient in these experiments to demonstrate this effect. It seems that this antibody-antigen combination was strong enough to resist degradation or separation in a remarkable degree. It is not clear why, by transfer of normal sera, the cellular antibody-formation has been suppressed.

Even after application of isologous normal serum local influence was observed; this must be discussed later. The phenomenon that transfer of normal serum of C 57 Bl-mice enhanced the cellular antibody-formation cannot be explained.

These findings show an influence of heterologous and homologous antisera on the cellular antibody-formation as well as of isologous antisera. This is the principle of a feedback-system; but it is yet not clear, if there is only a peripheric effect involving the antigenic sites or also a central effect involving the immunocompetent cells directly. Regarding this question it is of interest, that application of heterologous and homologous as well as of isologous immunserum did not influence the "background". If there is a cellular mechanism, the background might have been suppressed also.

Zusammenfassung

Unterdrückung der 19S- und 7S-Immunglobulin-Synthese durch heterologe und homologe Antiseren

Durch Sensibilisierung von Kaninchen, Meerschweinchen sowie Mäusen der Stämme C57Bl und AKR mit Hammelerythrozyten (HE) wurden Immunseren gewonnen. Diese wurden jeweils 1 Tag vor und nach der Sensibilisierung von AKR-Mäusen mit HE diesen Tieren verabreicht. 10 Tage später wurde die zelluläre Antikörperbildung mit der Plaque-Technik nach Jerne bestimmt. — Gegenüber sensibilisierten AKR-Mäusen ohne Serumapplikation war die 19S- und die 7S-Immunglobulinbildung in Milzzellen fast komplett unterdrückt, sofern Immunseren verabreicht worden waren. Applikation von Normalseren ohne Antikörper gegen HE zeigte nur einen geringfügigen Einfluß. — Die Ergebnisse beweisen, daß durch Übertragung heterologer und homologer Antikörper die Immunantwort ebenso unterdrückt werden kann wie durch Zufuhr isologer Antikörper.

Résumé

La suppression de la synthése du 19S- et 7S-immunglobulin par des immunsérums hétérologues et homologues

Du immunsérum fut produit par application des globules rouges du mouton (GR) aux lapins, aux cochons d'Inde et aux souris C57Bl et AKR. Les immunsérums ont été injectés un jour avant et après la sensitisation des souris AKR par des GR. Après 10 jours la production cellulaire d'anticorps contre les GR fut objectivée par la Jerne-plaque-technique. Par rapport aux animaux sensibilisés sans injection du serum la creation du 19S- et du 7S-immunglobulin fut supprimée presque totalement dans les cellules spleniques après application d'immunsérum. L'application des sérums normaux sans anticorps contre GR a diminué la creation du immunglobulin peu d'importance. Des immunsérums n'ont pas en aucune influence sur le «background». Les résultats ont démonstré que la response immunologique peut être supprimée soit par une transmission passive des anticorps hétérologoues et homologues, soit par une application passive des anticorps isologues.

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Lebenslauf

Ich wurde am 2. Oktober 1946 als viertes Kind des Kaufmanns Albert Schmid und seiner Ehefrau Maria, geb. Weidelener, in Bad Schussenried (Württ.) geboren. Von 1954 bis 1958 besuchte ich die dortige Volksschule und wechselte dann auf das Progymnasium über, das ich vier Jahre besuchte. Danach begab ich mich als Internatsschülerin in das Mädchengymnasium St. Hildegard in Ulm, an dem ich 1966 mein Abitur ablegte. Seit Sommer 1966 studiere ich an der hiesigen Universität Medizin.